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# *Lactobacillus rhamnosus* 271

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## Probiotics

Live bacteria which upon ingestion exert health benefits beyond inherent basic nutrition are called probiotics. Different strains of *L. rhamnosus* has for long been used as probiotics in a wide range of different probiotic products, marketed in many countries. The most well known strain is *Lactobacillus rhamnosus* GG (Gorbach and Goldin 1992; Alander *et al.* 1999). This particular strain of *L. rhamnosus* has been proved to have several health beneficial effects. Most well documented is that *L. rhamnosus* GG shortened the duration of diarrhoea in acute rota virus gastroenteritis, probably by stabilising the intestinal mucosa and promoting immune response (Majamaa *et al.* 1995; Huang *et al.* 2002). Also well documented is that *L. rhamnosus* GG have been successfully used in the treatment of recurrent *Clostridium difficile* colitis by reducing the frequency of diarrhoea and preventing relapses (Bennet *et al.* 1996; D'Souza *et al.* 2002).

The bacterial strain, *Lactobacillus rhamnosus* 271 (= DSM 6594), has been included in different types of yoghurts or similar products based on milk. The bacterium is added as probiotics but is also growing in the product during manufacturing and by its activity improving taste and consistency of the product. *L. rhamnosus* 271 has been included in several commercial yoghurt products through the years [for example, Viktväktarnas yoghurt (Skånemejerier, Malmö, Sweden), and Fundo<sup>TM</sup> (Milka, Finland)]. The concentration of *L. rhamnosus* 271 in commercial products has usually been around  $5 \times 10^7$  colony forming units [CFU] per ml.

The strain, *Lactobacillus rhamnosus* 271, is granted patent in Europe and USA, amongst others. Possessor of all rights are Probi AB, Lund, Sweden).

## The species *Lactobacillus rhamnosus*

*L. rhamnosus* is a bacterial species in the huge and relatively diverse genus of *Lactobacillus*, which comprises about 90 validly named species. *L. rhamnosus* is a so called facultatively heterofermentative *Lactobacillus*, i.e. *L. rhamnosus* ferments hexoses exclusively to lactic acid, but can also ferment pentoses and/or gluconate, and then producing lactic and acetic acid. The type strain of *L. rhamnosus* is ATCC 7469.

Striking characteristics of *L. rhamnosus* are:

- 1) *L. rhamnosus* grow rapidly in milk.
- 2) *L. rhamnosus* often possess an ability to produce extra-cellular poly-saccharides (slime).

The species *L. rhamnosus* is frequently present on human gastro-intestinal (GI) mucosa of healthy individuals (Molin *et al.* 1993; Ahrné *et al.* 1998), and was the dominating *Lactobacillus* species in Swedish breastfed children (Ahrné *et al.* 2005).

It has been shown that different strains of heat-killed, whole cells of *L. rhamnosus* were more efficient in triggering the production of the regulatory cytokin IL-10 in human blood mononuclear cells (monocytes), *in vitro*, than were cells of *Lactobacillus plantarum* or *Lactobacillus paracasei* (Hessle *et al.* 1999).

## The strain, *Lactobacillus rhamnosus* 271

*L. rhamnosus* strain 271 (= DSM 6594) has been isolated from healthy human colonic mucosa (Molin *et al.* 1993).

*L. rhamnosus* 271 can be defined and identified by restriction endonuclease analysis (REA) of total chromosomal DNA by the use of relatively frequently cutting restriction enzymes such as *EcoRI* and *ClaI*, and traditional agarose gel electrophoresis (Johansson *et al.* 1995). This method was successfully used for strain-definition and re-isolation of *L. rhamnosus* 271 from mucosal biopsies obtained in an administration study in humans (Johansson *et al.* 1993). *L. rhamnosus* 271 could be re-isolated from intestinal biopsies after oral administration of the strains (Johansson *et al.* 1993). In one individual *L. rhamnosus* 271 could even be found as a major part of the mucosal lactobacilli-flora 11 days after the end of administration (Johansson *et al.* 1993).

Irrespective of what strain that is used as probiotics, an absolute condition must be that the bacterium survives and remains active during the passage through the gastro-intestinal tract. The ability of *L. rhamnosus* 271 when administrated in fermented milk products to survive the passage through the human GI-tract has been proved (Ahrné *et al.* 1995).

In a few individuals, the strain can remain for a week or two after ended consumption (Johansson *et al.* 1993; Ahrné *et al.* 1995).

*L. rhamnosus* 271 has *in vitro* shown to adhere to Caco-2 cells, survive a pH of 2.5 for 4h, and to tolerate 0.3% oxgall (Jacobsen *et al.* 1999).

*L. rhamnosus* 271 has shown to possess antimicrobial activity *in vitro* against strains of potentially pathogenic species as *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Shigella flexneri*, *Yersinia enterocolitica*, *Citrobacter freundii*, *Enterobacter cloacae* and *Enterococcus faecium* (Jacobsen *et al.* 1999).

## Experimental studies on rats

The translocation, i.e. the passage of viable bacteria through the epithelial mucosa into the *lamina propria* and then to the mesenteric lymph nodes and possibly other tissues (Berg and Garlington, 1979), can be slightly reduced in rats with acute liver injury (induced by D-galactoseamine; Kasravi *et al.* 1996a and Kasravi *et al.* 1996b) by the administration of

LR271 (Adawi *et al.* 1997). Daily rectal supplementation of *L. rhamnosus* 271 for 8 d before liver injury decreased the incidence of bacterial translocation to the arterial blood 24 h after liver injury (Adawi *et al.* 1997).

Reduced translocation was also seen in a methotrexate-induced enterocolitis model when the rats along with the methotrexate-treatment were given *L. rhamnosus* 271 (Mao *et al.* 1997). Supplementation of *L. rhamnosus* 271 significantly lowered the bacterial translocation to mesenteric lymph-nods, liver and aortic blood. This decrease in translocation was accompanied by a reduction in the total load of bacteria and the number of *Enterobacteriaceae* in the ileal and cecal content. The gut mucosal protein, DNA and RNA content were significantly increased by the supplementation with *L. rhamnosus* 271 (Mao *et al.* 1997).

## An unpublished trial on IBS

The effect of a yoghurt-like product, containing *L. rhamnosus* 271, on the experienced abdominal pain, flatulence and stool function of patients with irritable bowel syndrome (IBS) was evaluated in a placebo controlled, double blinded study (Nobaek *et al.* 2000; the study was run 1998 but has never been published). Patients were divided into two treatment groups and a placebo group. One treatment group was administered *L. rhamnosus* 271 together with *Lactobacillus acidophilus* SKB3 in a rather low concentration in milk fermented with *Streptococcus thermophilus* SKB3 (RA-group), and the other treatment group was given LR271 in fermented milk (R-group). The placebo group (P-group) was given the *Str. thermophilus* fermented milk. Patients consumed the different products for 28 d, 400 ml per day. RA and R products contained  $5 \times 10^7$  colony forming units (CFU) of *L. rhamnosus* 271. RA-group also contained  $1 \times 10^5$  CFU *L. acidophilus* SKB3 per ml, i.e. >100 times lower than the dose of *L. rhamnosus* 271.

A general problem with the study (Nobaek *et al.* 2000) was that there were patients with positive discoveries of *L. rhamnosus* 271 that was not supposed to harbour *L. rhamnosus* 271. *L. rhamnosus* 271 was found in three persons before treatment (one person in the RA-group; two persons in the R-group) and in 10 persons consuming placebo. These persons were excluded from the final evaluation as they, against their instructions, obviously had been consuming a commercially available product containing *L. rhamnosus* 271 (for example, *L. rhamnosus* 271 was included in a yoghurt with the brand name Prima Liv, marketed by Slånemjerier). This commercial product was unfortunately, most probably, known to the participants. Thus, the efficacy evaluation included 32 persons in the RA-group, 32 persons in the R-group and 22 persons in the P-group (when the study was sent to a scientific journal for publication, this exclusion of participants was the main criticism of the study and presumably the main course for rejection).

LR271 was found in faeces and in rectal mucosa of most patients in the RA and R groups. The viable count of lactobacilli in faeces was constant in all three groups throughout the study. There was a trend in all groups that the viable count of *Enterobacteriaceae* from the rectal mucosa was higher 15 d after the end of the administration period than before, statistical significance being reached in the RA-group. The count of Gram-negative anaerobes and sulphite reducing clostridia showed a tendency to increase by the administration, statistically significant for the R-group and RA-group, respectively. The

concentration of succinic acid in faeces significantly increased in the treatment groups, while isovaleric acid increased in the P-group. No changes were shown in total concentration of carboxylic acids. Significant improvements in the stool function and flatulence were seen in all groups, but the decrease in flatulence was more pronounced in the RA and R groups. Abdominal pain was significantly reduced in the RA and R groups while the decrease in mean value for the placebo group failed to reach statistical significance. It is suggested that *L. rhamnosus* 271 counteract pain in IBS-patients by affecting the activity or composition of the intestinal bacterial flora (Nobaek *et al.* 2000).

## Safety

*L. rhamnosus* 271 has been evaluated in the EU funded PROSAFE project (Vankerckhoven *et al.* 2008). The identity of the strain was confirmed and no acquired antibiotic resistance could be detected (PRO SAFE report on strain *Lactobacillus rhamnosus* 271).

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